CURRENT LITERATURE

NOTES FOR STUDENTS

Photosynthesis.—We are indebted to WILLSTÄTTER and his co-workers[1] for a series of contributions on the chemistry of the chloroplast and the mechanism and behavior of the photosynthetic apparatus. It is a promising sign that so eminent a chemist as WILLSTÄTTER recognizes that the chemistry of the cell is not the chemistry of the test tube and flask of the "true chemist," but rather the chemistry of a highly complex colloidal substrate with its enzymes. Physiologists can well look forward with keen anticipation to further notes from his laboratory, since we have this statement concerning his future work: "Analyse physiologisch bedeutsamer Stoffe ist nicht Selbstzweck, sondern Vorbereitung für physiologische Forschung."

The leaf pigment chlorophyll is a mixture of two green pigments, a blue-green (chlorophyll *a*) and a yellow-green (chlorophyll *b*), and two yellow pigments, carotin and xanthophyll. In the Phaeophyceae a third carotinoid (fucoxanthin) is found. The chlorophylls of all plants investigated (almost 200) are neutral substances carrying a complex magnesium compound whose molecule is built up of 4 freely substituting pyrrol nuclei and are of the following compositions respectively: $(C_{31}H_{29}N_3Mg)$ (CONH) $(COOCH_3)$ $(COOC_{20}H_{39})$ and $(C_{31}H_{27}ON_3Mg)$ (CONH) $(COOCH_3)$ $(COOC_{20}H_{39})$. These compositions were determined by a judicious and cautious treatment of chlorophyll extracts with proper alkalies and acids in proper concentrations. It was found that splitting with acid made it possible to preserve and find in the products of hydrolysis that compound which is separated by alkalies, and, conversely, that the alkali derivatives exhibited a characteristic group which is destroyed with extraordinary ease by acids. Saponification of the pigment with alkali yields green, water soluble salts, the chlorophyllins. Progressive removal of

[1] WILLSTÄTTER, R., and STOLL, A., Untersuchungen über Chlorophyll. 8vo. pp. viii+424. *pls. 11.* Berlin: Julius Springer. 1913; see review of recent work on the pigments of the green leaf and the processes connected with them in New Phytol. **14**:281–294. 1915.

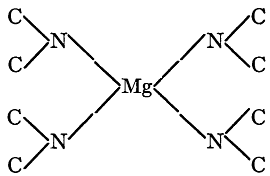
———, Über die chemischen Einrichtungen des Assimilationsapparates. Sitzb. Kgl. Preuss. Akad. Wiss. **20**:322. 1915.

———, Über die Assimilation ergrünender Blätter. Sitzb. Kgl. Preuss. Akad. Wiss. **36**:523. 1915.

———, Untersuchungen über die Assimilation der Kohlensäure. Ber. Chem. Gesells. **48**:1540–1564. 1915.

WILSTÄTTER, R., Sitzb. Kgl. Preuss. Akad. Wiss. **32**:484. 1915.

the carboxyl groups with alkali yields a series of phyllins, the last oxygen free one being aetiophyllin, which carries the Mg group intact, has the composition $C_{31}H_{34}N_4Mg$, and hypothetically the structural nucleus



Acid treatment of the phyllins removes the Mg and yields the porphyrins, aetioporphyrin ($C_{31}H_{36}N_4$), the equivalent of aetiophyllin, being the last of the series.

Treatment of chlorophyll with acids attacks another group, no saponification takes place, and a waxlike Mg-free chlorophyll derivative, phaeophytin, is obtained. Its solution differs in color from that of chlorophyll, but the green returns upon introduction of a metal, such as Zn, Cu, or Mg. Saponification of phaeophytin with alkali yields nitrogen-containing acids and a nitrogen-free alcohol phytol ($C_{30}H_{39}OH$), which may constitute one-third of the weight of chlorophyll and varies widely in amount in various chlorophylls. Chlorophyll is associated with an enzyme, chlorophyllase, active in alcoholic media, which causes an alcoholysis of the pigment and yields the chlorophyllides, the crystalline chlorophyll of the literature, according to the equation

$$\underbrace{(C_{32}H_{30}ON_4Mg)\ (COOCOC_3)\ (COOC_{20}H_{39})}_{\text{chlorophyll } a} + C_2H_5OH \rightarrow \underbrace{C_{20}H_{39}OH}_{\text{phytol}} +$$
$$\underbrace{(C_{32}H_{30}ON_4Mg)\ (COOCH_3)\ (COOC_2H_5)}_{\text{ethyl chlorophyllid } a}.$$

Plants can be divided into chlorophyllase-rich, such as *Heracleum Spondylium*, and chlorophyll-rich, such as *Urtica* sp.

The substance originally named phaeophytin by WILLSTÄTTER always yielded two distinct groups of decomposition products, one olive green in solution, the phytochlorins, the other red, the phytorhodins. Phytochlorin *e*, typical of the former series, is a tricarboxylic acid with two free carboxyl groups and one bound as a lactam; while phytorhodin *g* is a tetracarboxylic acid, only two or three of the carboxyls being in the free state. They are separated by a method based upon their different solubilities in ether and hydrochloric acid. Determination of the molecular weight of these substances, their occurrence in definite weight proportions, and their non-interconvertibility convinced WILLSTÄTTER that phytochlorin and phytorhodin arise from separate and distinct phaeophytins (*a* and *b* respectively), and that these in turn are derived from two distinct chlorophylls (chlorophyll *a* and *b*). These chlorophylls are obtainable in microcrystalline form and differ somewhat in their solubilities in various reagents.

The extraction of the chlorophylls can be carried out with dried or fresh material and is based on the use of solvents containing a moderate content of water, the latter forming salt solutions with some of the cell contents which alter the condition of the chlorophyll and render it soluble in the organic solvent. Separation and purification of chlorophyll *a* and *b*, carotin, and xanthophyll is based on their differing solubilities in solvents. Little is known of the chemistry of the carotinoids. They are hydrocarbons, both give non-fluorescent solutions, stable toward alkali but easily dissociated by acids, and both undergo auto-oxidation. Carotin and xanthophyll have the formulae $C_{40}H_{56}$ and $C_{40}H_{56}O_2$ respectively.

$$(C_{31}H_{29}N_3Mg)(CONH)(COOCH_3)(COOC_{20}H_{39}) \xrightarrow{\text{With acids}} (C_{31}H_{31}N_3)(CONH)(COOCH_3)(COOC_{20}H_{39})$$

chlorophyll *a* → phacophytin *a*

With alkalies ↓

$$(C_{31}H_{29}N_3Mg)(CONH)(COOH)(COOH) \longrightarrow (C_{31}H_{31}N_3)(CONH)(COOH)(COOH)$$

chlorophyllin *a* and isochlorophyllin *a* → phytochlorin *e* and phytochlorins *f* and *g*

↓

intermediate phyllines ⟶ intermediate porphyrins

$$C_{31}H_{34}N_4Mg \longrightarrow C_{31}H_{36}N_4$$

aetiophyllin → aetioporphyrin

WILLSTÄTTER and STOLL reinvestigated the question of the relation of chlorophyll content to photosynthetic activity of leaves in normal, chlorophyll-poor, vernal, and autumnal leaves. They used a modified Kreussler method and determined the assimilation number, $\frac{CO_2 \text{ assimilated in 1 hour (in gm.)}}{\text{chlorophyll (in gm.)}}$. They find not only that chlorophyll is absolutely essential for photosynthesis, but also that it acts in conjunction with a second indispensable inner factor, an enzyme, which has its seat in the contact zone between plastid and plasma. If the chlorophyll suffers more because of external factors, the assimilation number rises; if the enzyme suffers more, the number falls. The enzyme occurs in greatest proportion in juvenile, greening, etiolated leaves and in yellowish green leaves, and in all of these illumination is the limiting factor, while temperature is the limiting factor in chlorophyll-rich leaves in the photosynthetic process. Conclusions as to various steps in assimilation are drawn from the behavior of isolated chlorophyll as well as non-illuminated leaves toward carbon dioxide. Chlorophyll is not constantly decomposed and regenerated, as asserted by STOKES, neither do chlorophyll *a* and *b*, nor carotin and xanthophyll change into each other to any extent, as assumed originally by WILLSTÄTTER. The ratio $Q\frac{a}{b}=3$ is about constant under all conditions, though the ratio $Q\frac{c}{x}$ falls during the course of extended experiments. A striking difference was noted in the behavior of chlorophyll towards CO_2 when

in true solution in ether or alcohol, and in colloidal solution, as watery acetone. The former is exceedingly stable toward alkali and acids, while the latter is stable toward alkali, but so sensitive to acids that CO_2 splits it readily into $Mg(H\ CO_3)_2$ and phaeophytin ($C_{55}H_{72}O_5N_4Mg + 2CO_2 + 2H_2O \rightarrow Mg\ (H\ CO_3)_2 + C_{55}H_{74}O_5N_4$). Such a solution loses half of its metal in 4 days when treated with 5 per cent CO_2. Before the Mg is displaced, however, an intermediary dissociable compound is formed, maximum in quantity at low temperatures. The time factor is much more active in the chlorophyll *a* than in the other compound. Leaves are thus endowed with a mechanism for storing CO_2 much as the blood is. Instead of the albumin of the serum holding the CO_2, however, it is possible that carbamino compounds of amino acids or proteins are involved here. This dissociable CO_2 chlorophyll compound may take up the light energy and give an isomer of greater energy content which is suitable for spontaneous decomposition. This may be the substance that is split enzymatically.

Interesting in this connection is a paper by SPOEHR.[2] In an excellent critical review of the literature he points out certain facts that seem to weigh heavily against the theory of formaldehyde as an intermediate product in photosynthesis and in favor of formic acid as such. Discussing the work of LOEB with CO_2 and silent discharge, USHER and PRIESTLY, BERTHELOT and GUADECHOW, and STOCKLASA with CO_2 and ultraviolet light, he concludes that we have no clear record of a reduction of carbonic acid on its salts to formaldehyde which is applicable to the process going on in the plant leaf or is compatible with conditions met in the leaf. NEF'S success in condensing formaldehyde to sugar might be of significance, he believes, if an enzyme could be substituted for the strong alkali and high temperature used. WILLSTÄTTER'S work shows clearly that enzymes and the colloidal state of chlorophyll and the plasma are important factors in this process. It also shows that the greatest progress in physiology is to be made by work that combines chemical and physiological technique judiciously. SPOEHR finds that while formaldehyde yields sugars in light or dark only in the presence of relatively strong bases, such as do not exist in the plant, CO_2 in aqueous solution in ultraviolet light produces formic acid as the only product, and exposure of this to sunlight or ultraviolet light gives a substance having several properties and the nutritive value of sugars. He believes formic acid may well be the intermediary product of photosynthesis, as suggested by ENGELMANN, especially since organic acids in sunlight give rise to formaldehyde and vice versa. The formaldehyde reported in green leaves, as shown by WAGER and WARNER, may be due to the activity of light on chlorophyll and other plant substances. SPOEHR feels that the findings of GRAFE, BOKORNY, and BAKER, that formaldehyde is assimilated by the green leaf only in presence of sunlight, introduces an entirely new

[2] SPOEHR, H. A., The theories of photosynthesis in the light of some new facts. Plant World **19**:1–16. 1916.

factor into the BAEYER hypothesis, to which altogether too much attention has been paid by chemists and physiologists.

The reviewer feels that the results of the chemist WILLSTÄTTER, who, to paraphrase a statement of JOHANNSEN'S, carries on his physiological studies not as chemistry, but with chemistry, deserve the notice of all physiologists and chemists, and are full of promise of a new attack upon the most fundamental material problem of mankind, namely, the increase of the food supply of the world.—G. K. K. LINK.

The bubble method in photosynthesis.—KNIEP[3] has made a critical study of the value of the bubble method for comparing the rate of photosynthesis in water plants under various conditions. From his work it is evident that the method must be applied with great caution. Part I deals with the percentage of oxygen in eliminated gas bubbles. This percentage rises as the rate of bubbling increases, but not in direct proportion. If one were to find the total oxygen production due to photosynthesis, he would need to determine (1) the volume of the eliminated gas with its percentage of oxygen, and (2) the amount of oxygen that diffused into the bathing water. For the analysis of eliminated gas KNIEP used Krogh's apparatus, by which the percentage of oxygen and carbon dioxide can be determined in very small samples of 4–6 $mm.^3$ The amount of oxygen diffusing into the bathing water was determined before and after a period of photosynthesis by WINKLER'S titration method. The percentage of oxygen in the eliminated gas varied from 22.8 per cent in slow photosynthesis to 45 per cent in rapid photosynthesis.

The whole matter is complex. The oxygen production in photosynthesis not only increases the volume of gas in the intercellular spaces and leads to elimination of gas from the cut surface of the stem, but it increases the oxygen percentage in these spaces and thereby steepens or sets up diffusion gradients (falling gradient of oxygen from intercellular spaces to bathing water and of nitrogen in the opposite direction). Carbon dioxide diffusion gradients due to its consumption must be considered also. Hence the amount of gas given off as bubbles and its richness in oxygen and other gases is the resultant of all these processes acting together, a given set of conditions giving one equilibrium and a change in conditions gradually leading to another set of conditions.

Part II presents the influence of water movement upon the bubble outgo. With 1 per cent $KHCO_3$ in distilled water as the bathing medium, water movement temporarily stops the bubble outgo. With tap water (supersaturated) the outgo of bubbles increases and continues in darkness even in dead plants. This behavior is all explained on the basis of the effect of the water movement upon the gas gradients. On the basis of these results KNIEP pointed out the

[3] KNIEP, HANS, Über den Gasaustausch der Wasserpflanzen. Ein Beiträge zur Kutik der Blazenzählmethode. Jahrb. Wiss. Bot. **56**:460–509. 1915. PFEFFER'S Festschrift.